Chemically contaminated drinking water sources provide ways of exposure for many potential environmental health hazards. The pollution of drinking water sources by industrial and agricultural wastes is a widespread problem all over the world [1]. Many countries in the EU, Romania included, have problems in rural areas, where the networks are small or consumers depend on private wells, and treatment of drinking water is either poor or nonexistent [2].

Underground waters are still important sources for drinking water in our country, and a big part of our population are using these sources for alimentary and agricultural purposes [1]. Unfortunately, a lot of our wells waters are already polluted with nitrates and other industrial and farming chemicals. The composition and quality of water from underground sources depends on the quality and composition of soil, on the possible sources of contamination nearby and also on the quality of wells construction and sanitary conditions for protecting the water source [2, 3].

In our country the chemical composition of wells drinking water was less evaluated in publications in the last years, even it is known the relationship between high levels of nitrates from wells sources of drinking water and the methemoglobinemia (MHG) on infants exposed especially for those that are not breastfed by their moms.

A specific area of concern is depth well-water sources, the level of well-water nitrate consumed, the status of soil composition and pollution sources nearby) in order to evaluate the level of nitrates chemical pollution of underground water sources from different country regions.

Interventional outcomes: evaluation of risk exposure and frequency of blue infant illness in our country, followed by community intervention focused on decreasing of this disease frequency by local measures of drinking water quality improving and also by sustained health education among exposed population.

Experimental part

We designed our study from two overlay studies that have completed each other: the first was an analytical study based on laboratory tests made on underground water samples from 27 Romanian districts (with different soil composition and pollution sources nearby) in order to evaluate the level of nitrates from the water and the second one was a clinical convenience retrospectiv study regarding the frequency and trend of MHG illness registered in infants hospitalised in 2014 (data superposed over the same regions studied for nitrates composition in drinking underground water sources).

Keywords: underground water, nitrates, methemoglobinemia, fertilizer
Data regarding the level of nitrates from drinking water sources were gathered in 2013 by the regional Centers of Public Health Institution from all over the country (by national monitoring program performing spectrophotometric and volumetric methods), on different rural wells and by seasons and we completed with some results from tests made in the last two years at the University of Medicine and Pharmacy from Tîrgu Mureș labs from four districts nearby that were missing from the list (Mureș, Harghita, Covasna, Brașov and Salaj). In our lab we used a multiparametric colorimeter Hanna Instruments C99, and the tests were made based on the standard methodology required by our country [8-14], and the highest admitted limits permitted were upon the Romanian Law no. 458 from 2002 regarding drinking water quality and expertise (50 mg nitrates/L) [15].

Our data were statistically evaluated by using the Epi Info 6.0 program. The use of an exposure value made our analysis to be accomplished through univariate, relational chi-square tests, the likelihood ratio and Pearson tests, and also Wald chi-square statistic for comparison.

The second study was based on the continuous monitoring and registered data about cases of infants MHG from each hospital in the same regions we tested the rural wells water samples. The incidence of this illness was registered by number of cases reported on 10000 infants from the patient residency. Known cases of infantile blue illness meeting the recruitment criteria (a clinically diagnosed case with positive ascorbic acid response) and not exhibiting the exclusion criteria (hereditary MHG, medication- or dye-induced methemoglobinemia, early perinatal central nervous system damage, birth weight <2,000 g, and not speaking Romanian as a first language) were admitted to the study.

Results and discussions
The quality of well’s drinking water sources
We evaluated the statistical data from 27 Romanian districts (62.3% of all districts) who were monitored for the nitrates levels in the well’s water sources who induced MHG on infants (cases registered in 2013). It were individual wells 46% (registered in Bihor, Braila, Calarasi, Ialomita, Mures and Satu-Mare districts) but also community wells (in Olt, Vrancea, Iasi, Bacau and Vaslui); in Cluj, Covasna, Galati no wells and no cases of MHG were registered in 2013.

Most of the wells monitored for this situation were shallow wells, with depth less than 10 m and used like drinking water sources by the families (74%), but we had also deeper wells (more than 15 m) in Buzau, Galati and Vaslui districts.

Distance from pollution sources varied between 1 to 50 m, with high variance by districts (91%), with a median value of 14 m, and specifically lower in Covasna (1 m away), Neamț (2 m), Buzau (7 m), Olt (7 m) and Satu-Mare (8 m). The existence of protection source perimeter around the wells was recorded for all the wells monitored only in 5 from 26 districts (19.23%) and for the rest of them in a frequency between 50-60%, with a high risk of contamination with nitrates [16,17].

Regarding the presence of specific pollution sources like fertilizers or pesticides (who are involved in the pollution of soil from around the wells used for drinking water), we found out that 26.6% of water sources points had nearby natural fertilizers pollution sources ([las], Galati, Covasna, Cluj, Buzau, Bihor, Botosani), 3.8% artificial fertilizers (in Bihor, Prahova and Satu-Mare), 7.6% mixt fertilizers (Bacau, Bihor, Botosani, Suceava and Satu-Mare) and 1.3% insecticides sources (only in Suceava district).

The level of NO3- from water was related to the number of cases with MHG hospitalized: we found out that the nitrates tested under the standard limit of 50 mg/L transmitted the disease in 6 cases, the level of nitrates between 51-100 mg/L was identified to 15 cases of MHG (19% of all cases), the level of nitrates between 101-500 mg/L was identified in other 6 cases of MHG (7.6%), also for other 5 MHG cases (6.3% of all) the level of nitrates from drinking water was higher than 500 mg/L (found out especially in Moldova region). In Ardeal region, Salaj and Mures districts were the most contaminated underground water with nitrates from frequency point of view, but on average levels of nitrates (between 101-500 mg/L, 100.41 mg NO3-/L ± 38.06 C1) like other studies found [7,18].

Concentration of NO3- from water under 0.5 mg/L was detected in the most wells tested and for the most cases of MHG (78.5%) and for only 20% was > 0.5 mg/L.

More than 80% of controls and 79% of cases reported boiling water for infant formula preparation, good for bacteria killing but worse for nitrates concentration added [18, 19].

Epidemiological characteristics of MHG cases hospitalized in 2013
Annually incidence of those 82 MHG cases registered in 2013 was of 0.413/10000, meaning a high prevalence of MHG cases and higher than last years mentioned in other local studies [2,7,19], and also the incidence for infants group of age was 16.87/10000, with a pick in Iasi district (fig. 2).

Distribution of cases on districts showed that the highest frequency registered in Moldova historical region, on the East and North-East part of Romania, with 56.1% of all cases of MHG, also in rural parts of the country. We found out the highest frequency of intoxications in spring (33.6%) and summer seasons (28.2%) also in small infants from first three months years old. Regarding gender, we had a frequency higher at boys (58.6%) than girls but with no significance. Type of feeding on infants with MHG was mostly artificial (57.6%) than girls but with no significance. Type of feeding on infants with MHG was mostly artificial (57.6%) with significant predominence (p=0.0344) regardless the gender, and only 8.4% of them were breastfed.

The clinical severe cases were mostly on girls, with age under 6 months and no breastfeeding, and the moderate
clinical cases were more frequent on boys under 6 months old and also no breastfed. On 43% of cases acute diarrheal diseases were associated with MHG, like other studies mentioned to emphasize the symptoms of intoxication [8,18].

In our cases group studied, the individual values of haemoglobin varied between 6.60 to 18.80 mg/dL, with a big variance of series values on districts (21.17%). The smallest average values, under standard limits, and showing severe anemia, were registered in Calarasi (8.44 mg/dL), Vrancea (8.91 mg/dL), Vaslui (9.21 mg/dL) and Mures (9.80 mg/dL) (table 3).

From all the cases hospitalized, only half of them were tested for MHG (51.2%). Methemoglobin individual levels
continued

<table>
<thead>
<tr>
<th>District</th>
<th>Average</th>
<th>SD</th>
<th>Standard error</th>
<th>Confidence Interval</th>
<th>Min</th>
<th>Max</th>
<th>p</th>
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<tr>
<td>Bacau</td>
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<td>0.04</td>
<td>0.02</td>
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<td>3.63</td>
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<td>11.43</td>
<td>94.44 to 196.54</td>
<td>39.60</td>
<td>62.50</td>
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<tr>
<td>Salaj</td>
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<td>0</td>
<td>0 to 65.50</td>
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<td>65.50</td>
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</tr>
<tr>
<td>Iasi</td>
<td>33.21</td>
<td>22.33</td>
<td>4.99</td>
<td>22.76 to 43.66</td>
<td>3.00</td>
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</tr>
<tr>
<td>Neamt</td>
<td>45.29</td>
<td>0</td>
<td>0</td>
<td>0 to 45.29</td>
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<tr>
<td>Arges</td>
<td>16.50</td>
<td>0</td>
<td>0</td>
<td>0 to 16.50</td>
<td>16.50</td>
<td>16.50</td>
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</tr>
<tr>
<td>Mures</td>
<td>45.32</td>
<td>25.87</td>
<td>10.56</td>
<td>18.37 to 72.67</td>
<td>3.00</td>
<td>78.00</td>
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</tr>
<tr>
<td>Total</td>
<td>33.48</td>
<td>23.22</td>
<td>3.72</td>
<td>15.93 to 40.98</td>
<td>0.10</td>
<td>85.06</td>
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Table 4

<table>
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<tr>
<th>District</th>
<th>Average</th>
<th>SD</th>
<th>Standard error</th>
<th>Confidence Interval</th>
<th>Min</th>
<th>Max</th>
<th>p</th>
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<tbody>
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<td>Iasi</td>
<td>10.96</td>
<td>2.66</td>
<td>0.59</td>
<td>9.71 to 12.20</td>
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<td>7.67 to 13.54</td>
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<tr>
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<td>0.74</td>
<td>10.10 to 14.23</td>
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<tr>
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<td>1.07</td>
<td>0.53</td>
<td>9.33 to 12.72</td>
<td>9.67</td>
<td>12.03</td>
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<tr>
<td>Satu Mare</td>
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<td>-</td>
<td>-</td>
<td>- to -</td>
<td>13.40</td>
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<tr>
<td>Suceava</td>
<td>9.60</td>
<td>-</td>
<td>-</td>
<td>- to -</td>
<td>9.60</td>
<td>9.60</td>
<td></td>
</tr>
<tr>
<td>Teleorman</td>
<td>10.50</td>
<td>-</td>
<td>-</td>
<td>- to -</td>
<td>10.50</td>
<td>10.50</td>
<td></td>
</tr>
<tr>
<td>Vaslui</td>
<td>9.21</td>
<td>1.65</td>
<td>0.67</td>
<td>7.48 to 10.95</td>
<td>8.20</td>
<td>12.50</td>
<td></td>
</tr>
<tr>
<td>Vâlcea</td>
<td>8.91</td>
<td>-</td>
<td>-</td>
<td>- to -</td>
<td>8.80</td>
<td>8.80</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10.91</td>
<td>2.21</td>
<td>0.28</td>
<td>10.25 to 11.47</td>
<td>6.60</td>
<td>18.80</td>
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</tr>
</tbody>
</table>

varied between 0.23 to 87.3%, with a big variance of series of values (68.33%), with significant differences between districts evaluated (p<0.042).

The biggest average values, showing a severe intoxication, were registered in Galati (63.4%), Constanta (51%) and Neamt (45.3%) districts.

Also, we observed that on boys the anemia was more severe in association with MHG intoxication (r=-0.253), while in girls this parameters were independent (r=+0.054) (table 4).

Bacteriological characteristics of underground water used like drinking water showed positive tests for faecal coliformi more than 10/cm³ of water on 44% of samples and over 10 faecalis streptococcus/cm³ to 39.6% from MHG children hospitalized, like similar studies [7,18-20].

Conclusions

In the last years we have an important frequency of methemoglobinemia at infants in Romania, especially in some districts already knowned by their risk of exposure (like Moldova and Ardeal historical regions), with a decreasing in the last 3 years but no significant. The most exposed were infants in the first months after birth, especially boys, not breastfed, from rural areas, during spring time and even with severe forms of illness.

Testing the quality of underground drinking water sources involved in this type of intoxication we discover that most of infants were using water from community wells, with less than 10 m underground, close pollution sources like natural fertilizers, boiled before use and with high concentrations of nitrates.

We underline the importance of monitoring of underground drinking water sources from rural areas especially of those exposed to risk of high levels of nitrates in soil associated with evaluation of risk of exposure to nitrates upon infant population. Also education of population and preventive measures in these areas are very important, done by healthcare workers.

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11. *** Hotărâre nr. 974/2004 din 15/06/2004 pentru aprobarea Normelor de supraveghere, inspecţie sanitară şi monitorizare a calităţii apei potabile şi a Procedurii de autorizare sanitară a productiei şi distribuţiei apei potabile.
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